Elucidating the specific neuronal vulnerability in Friedreich ataxia preclinical models

Neurodegenerative diseases (NDs) remain an ever-increasing, incurable burden on our society, wherein specific neurons within defined regions of the central nervous system degenerate. To date, the mechanisms underlying the major NDs are poorly understood. One such group of neurodegenerative diseases is Hereditary Ataxias, caused by mutations in >60 genes, leading to the degeneration of cerebellar neurons and motor related circuits.

In our lab, we focus on Friedreich Ataxia (FA), an inherited early-onset ataxia with symptoms onset during adolescence, caused by a GAA repeat expansion within the frataxin gene, leading to low levels of frataxin (FXN). The disease onset and severity correlate to the residual levels of FXN, which inversely correlates with the length of the GAA expansion. FXN is a ubiquitous nuclear encoded mitochondrial protein involved in iron-sulfur (Fe-S) cluster biogenesis, an essential process in all cells that provide co-factors for the function of numerous proteins involved in key cellular pathways, such as central metabolism, protein translation and DNA metabolism. With disease progression, patients develop progressive ataxia of the limbs, associated with cerebellar dysarthria, sensory loss, and abnormal pyramidal signs, due to degeneration of proprioceptive neurons located in the dorsal root ganglia (DRG), a key component of the sensory system, the corticospinal tract, as well as the spinocerebellar tract, becoming wheelchair-bound within 10–15 years after onset. Interestingly, spinal motoneurons (MNs) are not particularly affected by the degenerating process until end stage of the disease unlike DRG sensory neurons (SNs). While much progress has been made in understanding the pathogenesis of FA through the development of animal and cell models, there are still many unanswered questions regarding the specific neuronal vulnerability, which might represent a breakthrough in the development of new therapies for patients.

The thesis project objective is to elucidate the specific neuronal vulnerability in FA, with two complementary aims:

Aim 1: To investigate the molecular differences underlying MNs and SNs vulnerability, we will take advantage of induced pluripotent stem cells (iPSCs) derived from FA patients with different GAA expansion sizes together with isogenic controls (where the expansion has been corrected using CRISPR/Cas9 system) and healthy controls. We will differentiate the iPSCs into spinal MNs and SNs. First, we will characterize the different cell lines in terms of expression markers of mature neurons, survival in culture, morphological analysis such as complexity of axonal breanching, soma size, electrophysiological properties using patch clamp, and mitochondrial activity. Finally, we will use omics techniques such as proteomics to identify new pathways that might be relevant for understanding neuronal vulnerability and the molecular signature that makes one type of neuron more vulnerable than the other in FA. With this approach, we will be able to identify specific pathways that can be relevant for the disease progression and that can be pharmacologically targeted.

Aim 2: This aim will be devoted to the characterization of MNs and SNs in a recently generated mouse model, FxnG127V KI (a point mutation previously found in FA patients), that presents low levels of frataxin in all cells types, recapitulating a scenario similar to FA-affected individuals. The phenotype in MNs and SNs in the FxnG127V KI mouse model has never been characterized before. Nevertheless, preliminary data from our team, show mitochondrial impairments and synaptic connectivity alteration in the cerebellum (another

major affected region in FA) that also reflect behavioural impairment. Therefore, we will characterize both dorsal root ganglia (DRG) and lower MNs longitudinally from pre-symptomatic to symptomatic stages of the disease from a morphological point of view, investigating mitochondria morphology and acitivity using enzymatic assay, synapses connectivity, and related activity markers. This deep characterization will allow us to identify the entry point to validate our human proteomics result in a more complex system and to have biomarkers that can be used as read out of a successful modulation of a specific pathway. To this aim the putative candiate pathway will be modulate via gene therapy using adeno-associated viruses (AAVs) or pharmacological approach in order to validate the relevance of the selected molecule in ameliorating FA pathophysiology both in vivo and in vitro.
This ambitious project tackles, for the first time, the relationship between vulnerable and resilient neurons in the context of FA. Previously, the absence of suitable disease models posed a significant obstacle to research progress. By integrating human iPSC models with a newly developed mouse FA rodent model, we aim to address a crucial question that is fundamental to FA research.

Supervisor(s) name(s), Affiliation(s), eMail address(es) for contact:
Hélène Puccio, Institut NeuroMyoGène – Pathophysiology and Genetics of Neuron and Muscle (INMG-PGNM) , UCBL-CNRS UMR5261 - Inserm U1315, Université Claude Bernard - Lyon I helene.puccio@inserm.fr

Proposed collaboration within ArchiFun network:
University of Padova Italy

Proposed list of secondments:
Prof. Elena Ziviani, Department of Biology, University of Padova, Padova, Italy

Main ArchiFun theme involved:

- ☐ Host-pathogen interactions;
- ☐ Mechanisms of bacterial resistance and cancer onsets;
- ☑ Neurodegenerative and autoimmune diseases;
- ☐ Translational research in prevalent diseases;
- ☐ Physiology and ecology;
- ☐ Neurosciences and cognition.